

## The Cytoplasmic Inclusions of *Nyctotherus macropharyngeus*: Histochemical Studies

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### SUMMARY

The cytoplasm of *Nyctotherus macropharyngeus* contains lipid bodies, mitochondria, and carbohydrate bodies. The lipid bodies consist of neutral lipids, most probably triglycerides, and protein. The mitochondria are rod-shaped. The carbohydrate bodies are formed by the coalescence of smaller spheres, which appear to arise in close association with the mitochondria. The external part of the smaller spheres is PAS-positive.

### INTRODUCTION

RICHARDSON and Horning (1931) described polymorphic 'Golgi bodies' in *N. cordiformis*, varying in shape from short rods to twisted filaments. In the same species Patten (1932) described certain twisted, snake-like objects as Golgi bodies. Khajuria (1950) gave the same name to granules in the cytoplasm of *N. macropharyngeus*.

Horning (1927) and Richardson and Horning (1931) described the mitochondria of *N. cordiformis* as rod-shaped; Patten (1932) described them as spherical granules. Khajuria (1950) described those of *N. macropharyngeus* as globular and rod-shaped.

'Vegetative granules' have been described in *Nyctotherus* by the authors named above. They appear as transparent spheres in the living organism, and are unstained by iron haematoxylin in fixed preparations. In the present paper they will be called carbohydrate bodies.

As far as the author is aware, no paper dealing with the histochemistry of the cytoplasmic inclusions of *Nyctotherus* is available. The purpose of the present work was to investigate, both morphologically and histochemically, the cytoplasmic inclusions of *N. macropharyngeus*.

### MATERIAL AND METHODS

Living specimens were examined in 0.85% sodium chloride solution by phase-contrast microscopy. Janus green B, neutral red, and other vital dyes were also used.

Specimens were placed in 2% osmium tetroxide solution and examined without further treatment.

The following fixatives were used for specimens that were to be sectioned subsequently: Lewitsky (strong Flemming without acetic), Regaud, Helly, Bouin, Carnoy. Paraffin sections were cut at 5  $\mu$ . They were stained either with iron haematoxylin (0.5% haematoxylin) or with acid fuchsine (Cain, 1948b).

Details of the histochemical tests used are given in the appendix (p. 521).

## OBSERVATIONS

In addition to the contractile vacuoles and food vacuoles, there are three distinct types of cytoplasmic inclusions: lipid bodies, mitochondria, and carbohydrate bodies.

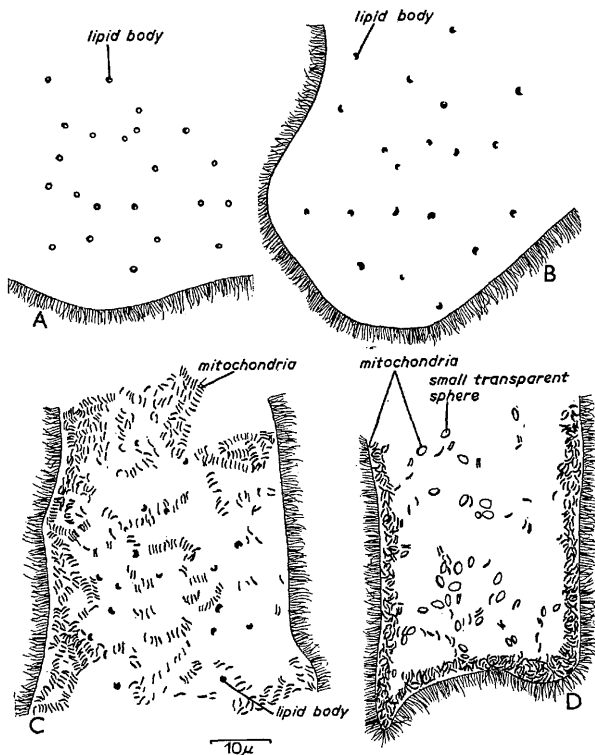


FIG. 1. Camera lucida drawings of portions of *N. macropharyngeus*. A, fresh material treated with 2% osmium tetroxide. B, formaldehyde-calcium, postchromed, Sudan black at 60° C. C, weak Bouin, pyridine extraction, mercuric bromophenol blue. D, Helly, Cain's acid fuchsin.

**Lipid bodies.** These are distributed at random in the medulla. Each appears to be duplex, consisting of externum and internum. The duplex structure is seen in the living organism and also after treatment with osmium tetroxide solution (fig. 1, A) and with Sudan black (fig. 1, B). The externum is darkened by the two latter techniques, while the internum remains clear. The externum appears crescentic in Sudan black preparations, and also in preparations

coloured by mercuric bromophenol blue (fig. 1, c). The latter result suggests the presence of protein in the externum. Ciaccio's 'unmasking' technique (see Gupta, 1958), followed by colouring with Sudan black, gives the same crescentic appearance as is seen in ordinary Sudan black preparations. The internum evidently contains no lipid; indeed, it did not give a positive response to any of the histochemical tests that were tried.

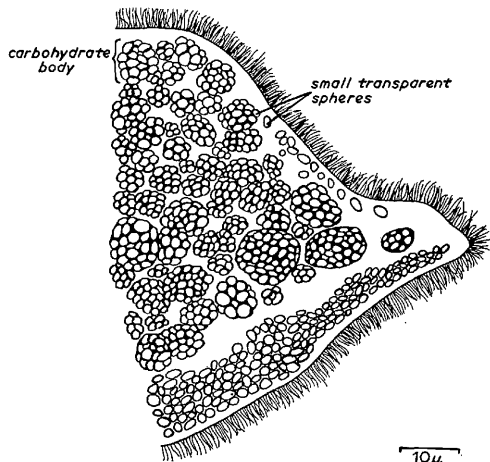


FIG. 2. Camera lucida drawing of a portion of *N. macropharyngeus*. Bouin, PAS. Only the outer parts of the small spheres are PAS-positive.

**Mitochondria.** These are rod-shaped. They are arranged in a peripheral layer just below the pellicle, and some are scattered also in the medulla. They are stained by mercuric bromophenol blue (fig. 1, c) and by acid fuchsin (fig. 1, d); they are also darkly stained by iron haematoxylin in material fixed in Lewitsky, Regaud, or Helly. They stain supravitaly with Janus green.

**Carbohydrate bodies.** Each of these is composed of numerous small spheres (fig. 2). The superficial part of each sphere is PAS-positive. The carbohydrate bodies give no positive response to tests for proteins or lipids, and are not stainable by neutral red during life.

The small spheres develop in close association with mitochondria (fig. 1, d). As each sphere grows, the associated mitochondrion begins to lose its affinity for acid fuchsin. Ultimately, a number of spheres coalesce to form larger bodies.

#### DISCUSSION

**Lipid bodies.** The evidence suggests that the externum consists of neutral lipid, probably triglyceride, and protein. The composition of the internum is unknown.

In *Opalina ranarum* the lipid bodies consist of triglyceride and lipoprotein; in *O. scalpriformis*, of lipoprotein only (Dutta, 1958). These two species and *N. macropharyngeus* live in the same environment, as gut parasites of frogs and toads, but their lipid bodies differ considerably in chemical composition.

The lipid bodies correspond to the so-called 'Golgi bodies' of earlier workers (Richardson and Horning, 1931; Patten, 1932; Khajuria, 1950).

The carbohydrate bodies or 'vegetative granules' were supposed by Horning (1927), Richardson and Horning (1931), and Khajuria (1950) to function as nutritional stores. It is possible that they develop under the influence of enzymes produced by mitochondria, as suggested by these authors.

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APPENDIX showing the various histochemical reactions of *Nyctotherus macropharyngeus*

Technique	Fixation	Embedding medium	Thickness of sections	Reference	Lipid bodies	Mitochondria	Carbo-hydrate bodies
SB in 70% ethanol	FCa and FCa + PC	G	5 $\mu$	Baker, 1946, 1956	++c	—	—
SB in 70% ethanol at 60° C.	"	"	"	Chiffelle and Putt, 1951	++c	—	—
SB in propylene glycol	"	"	"	Krishna, 1950	++c	—	—
SB* cold acetone	Fresh or FCa	"	"	Pearse, 1954	—	—	—
SB* cold ether	"	"	"	"	—	—	—
SB* cold ethanol	"	"	"	"	—	—	—
Sudan III and IV in 70% ethanol/acetone	FCa and FCa + PC	"	"	Kay and Whitehead, 1941	++c	—	—
Fettrot in 70% ethanol	"	"	"	Pearse, 1954	++c	—	—
Pink, Nile blue	"	"	"	Cain, 1947, 1948a	pink, ++c	—	—
Nile blue* cold acetone	Fresh or FCa	"	"	"	—	—	—
AH	FCa + PC	"	"	"	—	—	—
AH*PE	WB and PE	"	"	Baker, 1946	—	++	—
Fischer's reaction	FCa and FCa + PC	"	"	"	—	++	—
Feyrier's enclosure	FCa + PC	"	"	Pearse, 1954	blue	—	++
PAS	B, C, H, &c.	P	"	"	—	—	++
PAS* acetylation	"	"	"	Hotchkiss, 1948; Pearse, 1954	—	—	++
PAS* O.I.N. KOH	"	"	"	McManus and Cason, 1950	—	—	++
Performic acid / Schiff	"	"	"	McManus and Cason, 1950	—	—	++
MBB	FCa	G	"	Pearse, 1954; Lillie, 1952	++c	—	++
MBB*PE	FCa, FCa + PC, B, C, &c	G or P	"	Mazia and others, 1953	++c	++	—
Cholesterol reactions	WB and PE	G	"	"	++c	++	—
	FCa	"	"	Schulz, 1924, 1925; Pearse, 1954; Gomori, 1952; Romieu, 1927	—	—	—
Ciaccio's technique	FCa and phenol	"	"	Bradbury, 1956	++c	—	—
2% osmium tetroxide	Fresh	"	"	Nath, 1957	++r	—	—

KEY: AH = acid haematein; B = Bouin; C = Carnoy; c = 'crescent'; FCa = formaldehyde-calcium; G = gelatine; H = Helly; MBB = mercuric bromophenol blue; P = paraffin; PAS = periodic acid / Schiff; PC = with post-chroming; PE = pyridine extraction; r = rings; SB = Sudan black B; WB and PE = weak Bouin followed by pyridine extraction; + = weak reaction; ++ = moderate reaction; +++ = strong reaction; — = negative; \* = after treatment with.